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Occurrence of extended spectrum β-lactamase and AmpC genes among multidrug-resistant *Escherichia coli* and emergence of ST131 from poultry meat in Thailand

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ABSTRACT

This study investigated the prevalence of antibiotic-resistant *Escherichia coli* using extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase as exemplars of multidrug-resistant phenotypes in poultry meat samples taken from open-air and supermarkets in Phitsanulok province, Northern Thailand. Two hundred and fifty poultry meat samples from open-air ($n = 147$) and supermarkets ($n = 103$) were analyzed. In total, 143 cefotaxime-resistant *E. coli* isolates comprising 78 isolates (53.1%) from open-air markets and 65 isolates (63.1%) from supermarkets were obtained. No significant difference could be observed in the prevalence of ESBL-positive *E. coli* between samples taken from open-air (70.5%) and supermarkets (69.2%). ESBL genotypes comprised of $bla_{\text{CTX-M-group 1}}$ (69%), $bla_{\text{CTX-M-group 9}}$ (13%), $bla_{\text{TEM-116}}$ (1%), $bla_{\text{SHV-2a}}$ (1%) and $bla_{\text{SHV-12}}$ (1%) were detected. 39.5% of the ESBL-negative *E. coli* possessed $bla_{\text{CMY-2}}$. The $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 9}}$ and $bla_{\text{CMY-2}}$ were successfully transferred into *E. coli* by conjugation at high frequencies. Repetitive palindromic-PCR of some $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$-positive *E. coli* isolates revealed identical DNA patterns suggesting clonal spread. Phylogenetic grouping and MLST analysis revealed that 3 isolates were *E. coli* ST131. Of these, 2 isolates were ESBL-negative and carried $bla_{\text{CMY-2}}$. The other isolate was ESBL-positive and carried $bla_{\text{TEM-116}}$. This is the first study to demonstrate ESBL and AmpC genotypes in *E. coli* and the first discovery of human pathogen ST131 from Thai poultry meat. Our data raises serious concerns for food safety and biosecurity in the Thai food industry.

1. Introduction

Resistance to broad spectrum β-lactams such as third-generation cephalosporins, monobactam and carbapenems in Enterobacteriaceae, especially *Escherichia coli*, is rapidly increasing (Pitout, 2013). Reports on extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase-producing Enterobacteriaceae from clinical and environmental samples are continuously published from the majority of countries indicating a worldwide dissemination. Several types of ESBLs have been reported such as SHV, TEM and CTX-M. CTX-M is the most prevalent ESBL while CMY-2 is frequently encountered AmpC in human infections. ESBL- and AmpC-encoding genes are usually associated with mobile genetic elements which strongly facilitate their spread within a bacterial population (Jacoby, 2009; Poirel, Bonnin, & Nordmann, 2012).

Contamination of meat with antibiotic-resistant bacteria has the potential to transfer to humans and is a clear public health concern. Several studies have shown that meat, especially poultry meat, is an important reservoir of antibiotic-resistant *E. coli* (Nguyen et al., 2016; Schwaiger, Huther, Hölzel, Kämpf, & Bauer, 2012). The prevalence of ESBL- and AmpC-positive *E. coli* as well as their respective resistant genes in different types of meat even in organic meat has been reported from several countries (Cohen Stuart et al., 2012; Egea et al., 2012; Ghodousi, Bonura, Di Noto, & Mammina,

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2015; Kawamura, Goto, Nakane, & Arakawa, 2014). Furthermore, transmission of ESBL and AmpC-positive *E. coli* from meat to human has been previously reported outside Thailand (Overdevest et al., 2011; Vincent et al., 2010).

In Thailand, consumption of poultry meat is popular. Meat is often sold in open-air markets, traditional Thai markets which are seen extensively throughout the country, and western-style supermarkets which are becoming increasingly popular throughout Thailand.

Previous studies in Thailand have revealed the presence of antibiotic-resistant Enterobacteriaceae isolates in chicken meat and chicken rectal swab including those producing ESBL (Boonyasiri et al., 2014; Chaisatit, Tribuddharat, Pulsrikarn, & Dejsirilert, 2012; Trongjit, Angkittitrakul, & Chuanchuen, 2016). However, the prevalence of ESBL- and/or AmpC-producing *E. coli* from meat samples in Thailand remains poorly understood. This study investigated the prevalence of ESBL- and AmpC-encoding genes from poultry meat samples obtained from both open-air and supermarkets as well as determined *E. coli* pathogenicity groups.

2. Materials and methods

2.1. Samplings

Samplings of fresh poultry meat were performed in 29 open-air markets and 22 supermarkets in Phitsanulok province, Northern Thailand. A total of 250 poultry meat samples (chicken = 218, duck = 14, bird = 18) from open-air markets (n = 147) and supermarkets (n = 103) were sampled. Frozen poultry meat was excluded from the study. All samples were originated from Thailand. Samples were maintained at 4 °C and processed immediately.

2.2. Isolation and identification of third generation cephalosporin-resistant E. coli

Twenty-five grams of each sample were homogenized with a Stomacher in 225 mL buffered peptone water (Oxoid, Basingstroke, UK). Then, 10 mL of this homogenate were enriched in 90 mL EE broth (Becton, Dickinson and Company, MD, USA) for 24 h at 37 °C. The enrichment was plated onto EMB agar (Oxoid) supplemented with 2 μg/mL cefotaxime (as an exemplar of broad-spectrum cephalosporins) (Sigma Aldrich, MO, USA) and incubated under aerobic condition for 24 h at 37 °C. Presumptive *E. coli* colonies isolated from each sample were subcultured on Tryptic Soy Agar and incubated as described above for further characterizations. Species identification was performed by using RapID™ ONE System (REMEL Inc., KS, USA) according to the manufacturers' instructions and confirmed by sequencing of 16 S rRNA gene (Lane, 1991).

2.3. Antimicrobial susceptibility and ESBL detection

All isolates were tested for susceptibility to 18 antimicrobial agents by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) protocols and the results were evaluated according to CLSI criteria (CLSI, 2013). The antibiotics tested were ampicillin, cefoxitin, ceftazidime, cefotaxime, cefpodoxime, cefepime, aztreonam, imipenem, amoxicillin/clavulanic acid, ampicillin/sulbactam, amikacin, gentamicin, doxycycline, tetracycline, ciprofloxacin, levofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole. Isolates showing intermediate results were considered as resistant. Minimum Inhibitory Concentration (MICs) were determined by broth microdilution method according to CLSI guidelines (CLSI, 2013). The MIC of each antimicrobial agent was defined as the lowest concentration, which inhibited visible growth of the organism.

Isolates were tested for ESBL production by combination disk method with ceftazidime and cefotxime in the presence or absence of clavulanic acid, according to CLSI guidelines (CLSI, 2013).

2.4. Screening for ESBL- and AmpC-encoding genes by PCR and sequencing

All ESBL-producing isolates were investigated for the presence of ESBL-encoding genes; bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$, by PCR as previously described (Dallenne, Da Costa, Decré, Favier, & Arlet, 2010; Woodford, Fagan, & Ellington, 2006). Isolates showing resistance to cefoxitin were examined for the presence of AmpC-encoding genes by multiplex PCR (Pérez-Pérez & Hanson, 2002). PCR products were analyzed by agarose gel electrophoresis.

Selected PCR products were analyzed by DNA sequencing. Amplicons were purified using a DNA purification kit (RBC Bioscience, New Taipei City, Taiwan) and sequenced by First BASE Laboratories (Selangor, Malaysia). The obtained sequences were compared with those available in the GenBank database using the BLAST algorithm available on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

2.5. Conjugation experiments

To investigate the transfer of antibiotic resistance, conjugation experiments were carried out by broth mating method using rifampin-resistant *E. coli* DH5α as the recipient. Cultures of donor and recipient cells were mixed and incubated overnight at 37 °C without shaking. Transconjugants were selected on Tryptic Soy Agar supplemented with rifampin (16 μg/mL) and cefotaxime (1 μg/mL). Conjugation frequency was expressed as the number of transconjugants divided by the number of recipient cells. Transferrable of antibiotic-resistant gene was confirmed by PCR. MICs of transconjugants were determined by broth microdilution method.

2.6. Repetitive-palindromic polymerase chain reaction (Rep-PCR)

E. coli isolates carrying either ESBL- or AmpC genes were typed by rep-PCR as described previously by Versalovic, Koeuth, and Lupski (1991).

2.7. Phylogenetic grouping and multilocus sequence typing (MLST) analysis

Phylogenetic group (A, B1, B2 and D) of ESBL- and AmpC-producing *E. coli* was performed by a multiplex PCR assay for *chuA*, *yjaA* and DNA fragment TspE4C2 as previously described (Clermont, Bonacorsi, & Bingen, 2000). Isolate belonging to group B2 was investigated for the presence of *pabB* gene by PCR (Clermont et al., 2009). MLST was performed by amplification and sequencing of 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) according to the protocols from *E. coli* MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

2.8. Statistical analysis

Fisher's exact test was used to compare proportions using Minitab software version 15. The differences were considered statistically significant at $p < 0.05$.